

Abstract

Deletion of the *qmoABC* and a hypothetical protein (HP) (DVU0848-51) in the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough resulted in an inability to reduce sulfate. No suppressor mutations appeared in cultures of the deletion incubated in the presence of sulfate. Curiously, the $\Delta(qmoABC, HP)$ mutant was also unable to ferment pyruvate. Respiration of sulfate and fermentation of pyruvate was restored by supplying the *qmoABC*, HP genes. In order to verify if the hypothetical protein at the end of the operon contributes to the ability of this organism to reduce sulfate and ferment pyruvate, two complemented strains were constructed: both with the *qmoABC* genes, one with and one without the hypothetical protein. These two strains are compared for their ability to grow on sulfate as the sole electron-donor. Latest results suggest an integral role of the hypothetical protein in sulfate

Background (Figures 1a, 1b)

Two basic means to reduce sulfate: assimilative (used for amino acid synthesis in non-SRB) and dissimilative (used for sulfate respiration in SRB).

D. vulgaris contains the enzymes for both types of sulfate utilization (Fig. 1a). The operon containing the genes for dissimilative sulfate reduction, adenylylsulfate reductase, *apsBA*, is also predicted to contain the genes *qmoABC* (an electron transport carrier) and a hypothetical protein (Fig. 1b).

Sulfate Metabolism: Assimilatory and Dissimilatory

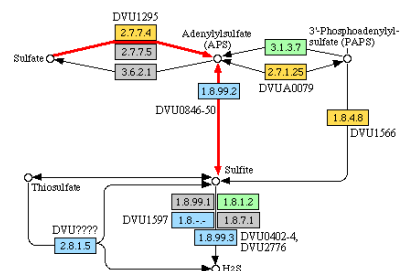


Figure 1a: Sulfate reduction genes in *D. vulgaris* and *E. coli*.



Figure 1b: Operon containing adenylylsulfate reductase genes, *apsBA*, *qmoABC*, and hypothetical gene (DVU0851) in *D. vulgaris*.

Last year's conclusions:

- Deletion of *qmoABC-hp* resulted in lack of ability to respire sulfate,
- complementation with an integrated copy of the complete operon, *apsBA* through the hypothetical protein encoding gene, was able to restore ability to respire sulfate.

Current question: Does the hypothetical protein (DVU0851) play a role in sulfate respiration?

Complementing plasmids (Figure 2)

Two stable plasmids (Fig. 2) were constructed to test complementation with or without the hypothetical gene (DVU0851). A non-native promoter (kanamycin promoter) was used to drive expression of the genes.

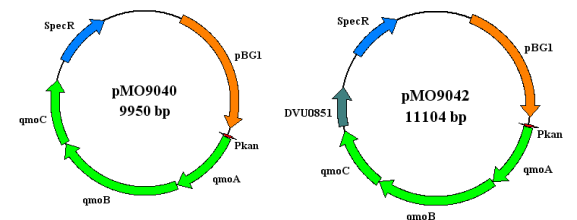


Figure 2: Diagram of complementing plasmids for $\Delta(qmo, hp)$ deletion.

pBG1: stabilization factor for plasmids within *Desulfovibrio*
Pkan: kanamycin constitutive promoter (from Aph)
SpecR: spectinomycin resistance gene, deletion mutant contains kanamycin resistance gene

Growth of complemented strains (Figures 3a, 3b)

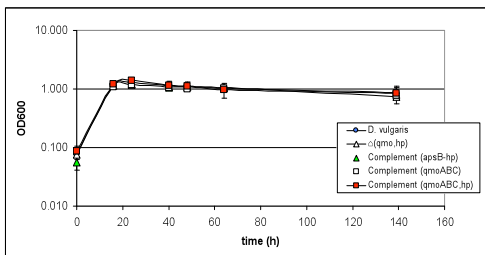


Figure 3a: Growth of *D. vulgaris*, $\Delta(qmo, hp)$, and three differing complementation constructs on lactate/sulfite (60mM/40mM, respectively).

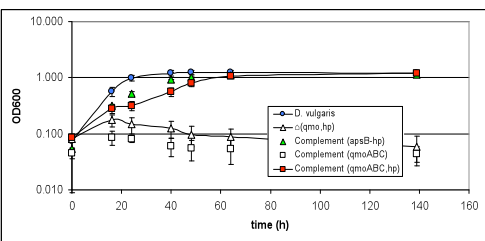


Figure 3b: Growth of *D. vulgaris*, $\Delta(qmo, hp)$, and three differing complementation constructs on lactate/sulfate (60mM/30mM, respectively).

Information about HP (DVU0851) (Figure 4)

Homologs of the hypothetical protein (DVU0851) can only be easily identified in the *Desulfovibrio* genus by their location (following the *qmoC* gene) but have limited conservation (Fig. 4).

Range of identity: 24% (DdeND132 vs. Dde27774) – 100% (DvH vs. DvDP4)

Range of similarity: 40% (Daf vs. Dde27774) – 100% (DvH vs. DvDP4)

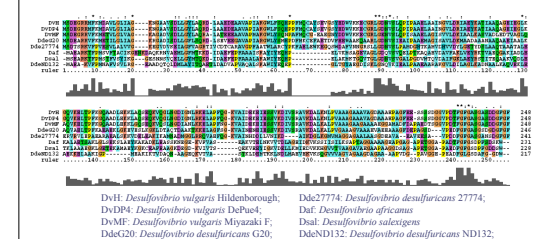


Figure 4: alignment of conserved hypothetical protein that follows *qmoC* in multiple SRBs.

No orthologs of DVU0851 have been identified in the following SRBs:

- *Desulforudis audaxiavor* (Gram-positive)
- *Desulfotomaculum reducens* (Gram-positive)
- *Desulfotalea psychrophila* (psychrophilic)
- *Archaeoglobus fulgidus* (Archaeal)
- *Syntrophobacter fumaroxidans* (oxidizes propionate syntrophically)

Only a few residues are strongly conserved: 10 of 200 amino acids.

Possibly important motifs: Fx₁₀G (amino-terminus)
LGx(N/Q)V (near the middle)
GGxPx₁₀(D/E)(T/D)FGFG (carboxy-terminus)

Conclusions

- Presence of the hypothetical protein is required for reduction of sulfate in *D. vulgaris*,
- The kanamycin promoter is expressed at a sufficient level to express the *qmoABC, hp* genes,
- DVU0851 is not highly conserved amongst the SRBs

Future work

- Delete hypothetical protein (alone),
- monitor for sulfate reduction,
- test complementation as necessary

ACKNOWLEDGEMENT

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